The kinome 'at large' in cancer

Emmy D. G. Fleuren[1], Luxi Zhang[2,3,4]*, Jianmin Wu[5] and Roger J. Daly[2,3]*

Abstract | Over the past decade, rapid advances in genomics, proteomics and functional genomics technologies that enable in-depth interrogation of cancer genomes and proteomes and high-throughput analysis of gene function have enabled characterization of the kinome 'at large' in human cancers, providing crucial insights into how members of the protein kinase superfamily are dysregulated in malignancy, the context-dependent functional role of specific kinases in cancer and how kinome remodelling modulates sensitivity to anticancer drugs. The power of these complementary approaches, and the insights gained from them, form the basis of this Analysis article.

Protein kinases regulate key processes such as cellular proliferation, survival and migration, and hence they are well poised to contribute to the various hallmarks of cancer if dysregulated. Indeed, 40 years ago, the first cellular proto-oncogene to be identified was found to encode a protein kinase, termed SRC[1]. Subsequent research has demonstrated key roles for many members of this enzyme superfamily, here referred to as the kinome, in human cancer development and progression, and has led to the development of highly selective and effective therapies directed against specific protein kinases that include BCR–ABL[2], mutant BRAF[3] and HER2 (also known as ERBB2)[4]. Many early discoveries relevant to the oncogenic role of protein kinases stemmed from candidate-based or positional cloning strategies. However, the latter have now been largely superseded by various 'omics' approaches that enable characterization of genetic alterations, protein expression and post-translational modification, as well as protein function, across the entire kinome, and thereby bring a global perspective to studies of protein kinases in cancer. Although each approach provides unique information, integration of data generated by more than one method often brings novel insights. Furthermore, interrogation of multi-dimensional data sets is leading to the development of network models of kinase regulation and function as well as novel therapeutic strategies. These exciting developments are discussed in this Analysis article.

Insights from cancer genomics

In 2004, the Cancer Gene Census (CGC), a literature-based census of genes that are mutated and causally implicated in cancer development[5] revealed that the most common protein domain encoded by cancer genes is the protein kinase domain, with 27 of 291 'cancer genes' encoding protein kinases[5]. Although this census highlighted the importance of protein kinases in cancer development, it pre-dated large-scale interrogation of cancer genomes. Consequently, it was likely that many protein kinases that represent oncogenic 'drivers' remained to be discovered, particularly those mutated at low frequency. Two studies lent support to this hypothesis. Sequencing of the coding exons of all protein kinases across 210 specimens of diverse human cancer types provided evidence, based on the ratio of non-synonymous to synonymous mutations in a particular gene, of driver mutations in approximately 120 protein kinases[6]. In addition, a survey of candidate cancer genes and those encoding 'druggable' targets across 441 specimens derived from various human cancers detected mutations in 157 of the 230 protein kinases studied[7]. Although these studies were published before the implementation of more stringent approaches for distinguishing between driver and 'passenger' mutation events[8], later studies incorporating such strategies supported the classification of some of the kinases identified — including activin A receptor, type 1B (ACVR1B), MAPK kinase kinase 1 (MAP3K1), AKT1, transforming growth factor-β receptor 2 (TGFβR2) and EPH receptor A6 (EPHA6) — as being encoded by cancer genes.

More recently, large-scale cancer genome sequencing efforts, including those involving The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), have greatly extended our knowledge of somatic genetic alterations characteristic of common human cancer types. These studies typically characterize hundreds of well-annotated clinical specimens of a given cancer type to great depth and thereby possess the power to reveal low-frequency mutations and the association of mutational events with particular subtypes of a particular cancer. In addition, they now employ algorithms that take into account factors such as the mutational burden for a given gene relative to background expectation, as well as the pattern of mutations

[1]*Department of Medical Oncology, Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, The Netherlands.*
[2]*Cancer Program, Biomedicine Discovery Institute, Monash University, Clayton, Victoria 3800, Australia.*
[3]*Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800, Australia.*
[4]*University of New South Wales, Sydney, New South Wales 2052, Australia.*
[5]*Cancer Division, Kinghorn Cancer Centre, Garvan Institute of Medical Research, 370 Victoria Street, Sydney, New South Wales 2010, Australia.*
**These authors contributed equally to this work.*
Correspondence to J.W. and R.J.D.
j.wu@garvan.org.au; roger.daly@monash.edu

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within that gene and its expression[8–10], in order to determine whether a gene is significantly mutated in a particular cancer and therefore the mutational event represents a likely driver. However, it should be noted that the majority of the analyses undertaken by TCGA and the ICGC were limited to exome, rather than whole-genome, sequencing. By definition, this approach will not detect mutations in other gene regions (for example, the promoter), and it also limits the ability of these studies to detect structural variants and gene fusions and to provide high-resolution analysis of copy number alterations (CNAs).

Despite these caveats, these studies have confirmed that certain well-characterized protein kinases are mutated at relatively high frequency (>10%) in one or more human cancers — for example, BRAF in thyroid cancer, skin cutaneous melanoma, multiple myeloma and colorectal cancer (60%, 51%, 15% and 10% respectively); epidermal growth factor receptor (EGFR) in glioblastoma and lung adenocarcinoma (27% and 12%, respectively); fibroblast growth factor receptor 2 (FGFR2) in endometrial cancer (13%); FGFR3 in bladder cancer (12%); Fms-related tyrosine kinase 3 (FLT3) in acute myeloid leukaemia (AML; 27%); and ERBB3 in bladder cancer (11%)[11–19]. However, some of these kinases are mutated at low rates in other cancers, which is of potential clinical relevance if targeted agents are available. For example, *BRAF* is mutated or subject to gene fusion events in 7% of lung adenocarcinomas and 3% of prostate cancers, respectively[16,20]. In addition, there is significant enrichment for mutations within certain key regulatory pathways in particular cancers. For example, in lung adenocarcinoma, genetic aberrations in the receptor tyrosine kinase (RTK)–RAS–RAF and PI3K–mTOR pathways can co-occur and characterize 76% and 25% of cases, respectively[16]. Furthermore, particular protein kinase mutations are associated with distinct molecular subgroups of a given cancer. For example, mutually exclusive loss-of-function mutations in MAP3K1 and MAP2K4, two consecutive kinases in the JUN N-terminal kinase (JNK) MAPK pathway, occur in approximately 20% of luminal A breast cancers[21], *JAK2* (encoding Janus kinase 2) gene amplification is associated with the Epstein–Barr virus-positive subgroup of gastric cancer[22], and *BRAF*-mutant thyroid cancers represent a distinct subclass from those harbouring RAS mutations, differing in cellular differentiation and gene expression signature[15].

A new census of protein kinase cancer drivers. Although a few cancer drivers are mutated at high frequency in one or more cancer types, many others are mutated at intermediate (1–10%) or low (<1%) frequency, and this 'tail' of lower-frequency mutations, together with the heterogeneity in mutation rate within and across tumour types, presents a challenge in generating a contemporary catalogue of cancer driver genes. However, the huge data resources generated by TCGA and other large-scale cancer genome sequencing studies, combined with stringent algorithms for detecting qualitative and quantitative patterns of mutation that highlight likely drivers, provide a powerful platform to begin addressing these issues. In addition, the ability to undertake 'pan-cancer' studies across multiple cancer types increases the power of the analysis, enabling detection of cancer drivers that would not be detected through interrogation of individual cancer types. In this Analysis article we have combined data from several high-powered, pan-cancer studies directed at identifying cancer driver genes in order to generate a new cancer gene census, and then extracted the protein kinases from this list, resulting in a list of protein kinase cancer drivers (Supplementary information S1 (table)), also publicly available at the Synapse platform, Accession No. syn4961550). Here, we have used the formally annotated complement of human protein kinases, which contains 518 members[23], but we acknowledge that additional enzymes with this activity have been identified, such as FAM20C[24]. In addition, the term 'cancer driver' is used to encompass both oncogenes and tumour suppressors. The studies used interrogated the following: somatic mutations across 12 (REF. 25) and 21 (REF. 10) major cancer types; somatic mutations, CNAs, fusion genes and expression data across 28 cancer types[26]; somatic mutations across >30 cancer types[27]; and mutations listed in the Catalogue of Somatic Mutations in Cancer (COSMIC) database, as well as cancer-associated gene amplifications, deletions and translocations[28]. In addition, we supplemented our list with additional protein kinases that are subject to gene fusion events, detected in a survey of fusion transcripts across 13 cancer types[29].

The list of cancer driver genes ($n = 1{,}100$) compiled from these studies is highly enriched for the presence of protein kinases ($n = 91$; $P < 2.2\text{e-}16$, Fisher's exact test). In addition, approximately 12% of non-kinase cancer drivers are substrates of kinase cancer driver genes, further highlighting the importance of kinase-regulated pathways in cancer. The CGC lists 41% of the protein kinase drivers (Supplementary information S1 (table)). The kinase drivers are distributed over all the various kinase subgroups (FIG. 1a), but there is a particularly strong representation from tyrosine kinases, which account for approximately 40% of the list. Some kinases, such as BRAF and EGFR, are drivers in multiple cancer types, whereas others are unique to specific cancers, such as KIT in AML and platelet-derived growth factor receptor-α (PDGFRα) in glioblastoma (FIG. 1b; Supplementary information S1 (table)). The most common type of genetic aberration is somatic mutation (single-nucleotide variants and small insertions and deletions), but CNAs and fusions also occur (FIG. 1a). Furthermore, the type of driver alteration in a certain kinase can differ between cancer types. For example, *FGFR3* exhibits mutations, CNAs and fusions in bladder cancer, CNAs or fusions in glioblastoma, and mutations in cutaneous melanoma (Supplementary information S1 (table)). Some driver kinases, such as ROS1, AKT3, insulin-like growth factor 1 receptor (IGF1R) and neurotrophic tyrosine kinase receptor type 3 (NTRK3), exhibit only oncogenic fusions or CNAs. Several kinases, including SRC, MAPK1 and MAPK8, were only reported as being drivers from the pan-cancer analysis, and were

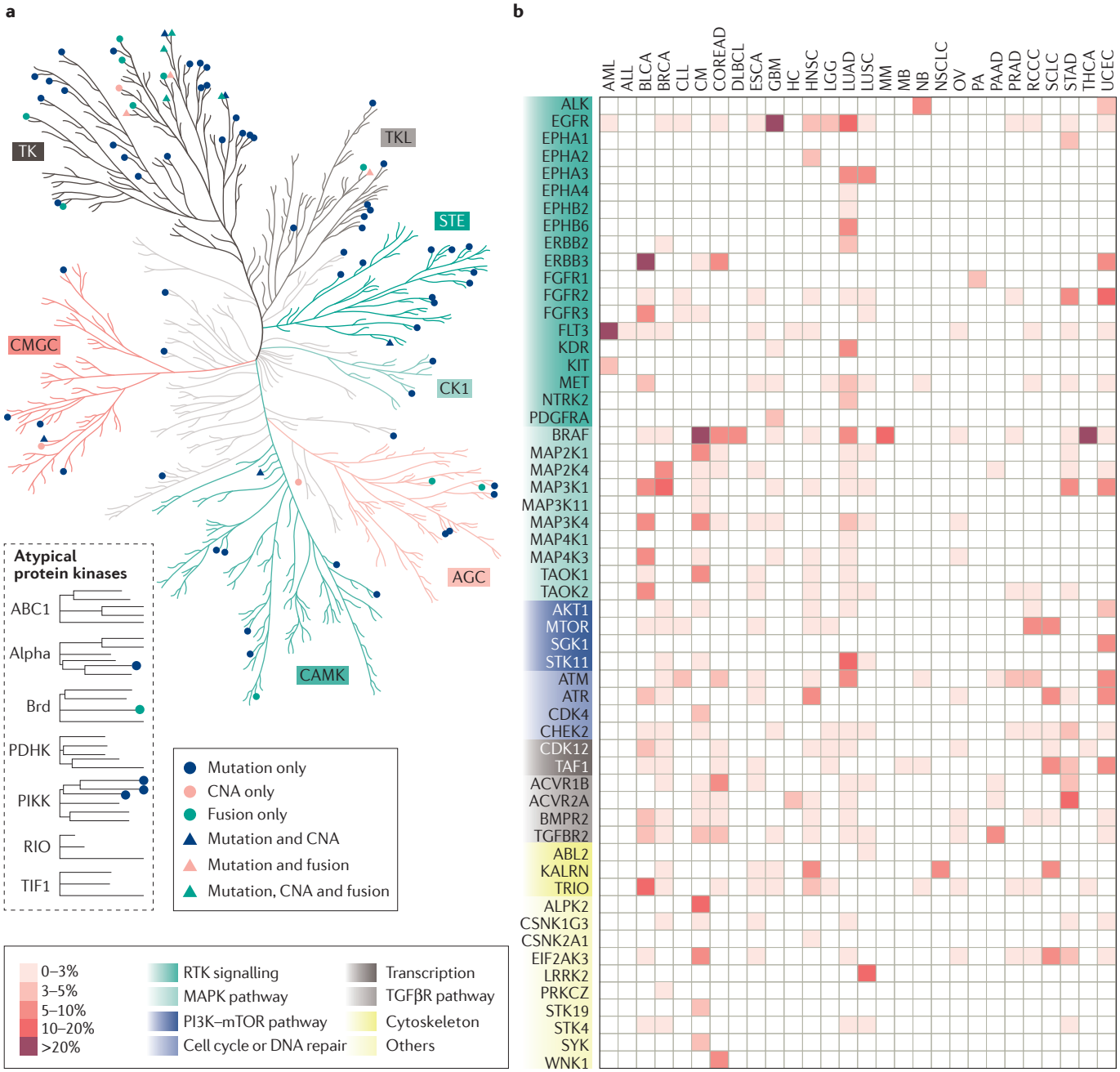


Figure 1 | **Driver protein kinases identified by genomic studies.** **a** | Driver kinases extracted from the cancer gene census generated for this article, and their corresponding genetic alterations in cancer. All 91 driver kinases are included, with the exception of TAF1 and STK19 (which could not be plotted). Kinome tree modified courtesy of Cell Signalling Technology Inc. (www.cellsignal.com) and annotated using Kinome Render[102]. The eight main kinase groups (including 'atypical') are highlighted: AGC (containing protein kinases A, G and C); CAMK (calcium/calmodulin-dependent protein kinase); CK1 (casein kinase 1); CMGC (containing cyclin-dependent kinase, MAPK, glycogen synthase kinase 3 and CDC2-like); STE (homologues of yeast sterile 7, sterile 11 and sterile 20); TK (tyrosine kinase); TKL (tyrosine kinase-like). **b** | Frequency distribution of kinase mutations in cancer types for which the corresponding kinase is classified as a driver. Percentages of samples mutated in individual tumour types are shown. The frequency data were extracted from major genomics studies used to create the kinase driver list [10,25,26] (Supplementary information S1 (table)). For kinases identified as a mutational driver in more than one study, the highest reported mutation frequency was selected. Assignment of the kinase to core signalling pathways or processes is also indicated. ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; BLCA, bladder carcinoma; BRCA, breast carcinoma; CLL, chronic lymphocytic leukaemia; CM, cutaneous melanoma; CNA, copy number alteration; COREAD, colorectal adenocarcinoma; DLBCL, diffuse large B cell lymphoma; ESCA, oesophageal carcinoma; GBM, glioblastoma; HC, hepatocellular carcinoma; HNSC, head and neck squamous cell carcinoma; LGG, lower grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MB, medulloblastoma; MM, multiple myeloma; NB, neuroblastoma; NSCLC, non-small-cell lung cancer; OV, serous ovarian adenocarcinoma; PA, pylocytic astrocytoma; PAAD, pancreatic ductal adenocarcinoma; PRAD, prostate adenocarcinoma; RCCC, renal clear cell carcinoma; SCLC, small-cell lung cancer; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrioid carcinoma.

PTEN
A phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase that antagonizes the PI3K signalling pathway and represents an important tumour suppressor in several human cancers.

not identified as drivers in any specific cancer type (Supplementary information S1 (table)). Certain malignancies, such as acute lymphocytic leukaemia and hepatocellular carcinoma, exhibit small numbers of driver kinases, whereas others, such as lung adenocarcinoma, are characterized by many (FIG. 1b). For cancers with large numbers of driver kinases, protein–protein interaction network analysis highlights how the mutated kinases exhibit connectivity and hubs corresponding to key cellular pathways (Supplementary information S2 (figure)).

Functional annotation of the identified kinase drivers. FIGURE 2 provides a schematic representation of the cellular pathways and processes regulated by the identified driver kinases, and Supplementary information S1 (table) contains a 'Detailed Information' tab that provides additional insights into their functional role in cancer. Almost one-half of the 58 known RTKs are among the identified kinases (FIG. 2a). These transmembrane proteins provide specific receptors for various hormones and growth factors, and regulate diverse cellular responses, including cell proliferation, survival and migration. Five transmembrane serine/threonine kinases that function as components of receptor complexes for specific TGFβ superfamily members are also present (FIG. 2b). Of these, ACVR1B, ACVR2A, TGFβR2 and bone morphogenetic protein receptor type 2 (BMPR2) exert tumour suppressor roles through specific mechanisms, including effects on cellular proliferation, migration and chemokine-mediated recruitment of myeloid cells[30,31]. Eight non-receptor tyrosine kinases are among the list, including specific members of the SRC and JAK families (FIG. 2c). Most of these kinases normally act to transduce and/or to regulate signals downstream of specific cell surface receptors that lack catalytic activity, such as particular cytokine receptors or integrins, or to augment signalling downstream of RTKs. However, ABL1 also functions in the DNA damage response[32]. There is strong representation from kinases that function in MAPK cascades that regulate proliferation, differentiation and stress responses (FIG. 2e). Of interest here is the involvement of MAPKs beyond those in the oncogenic RAS–RAF–ERK pathway (BRAF, RAF1 (also known as CRAF), MAP2K1 and MAPK1) to those that function in the p38 and JNK pathways. It is noteworthy that although the p38 and JNK pathway driver kinases each has a positive role in signalling within that pathway, they can have either tumour suppressor (for example, MAP3K1 and MAP2K4) or oncogenic (for example, MAPK8) roles, presumably reflecting the ability of these pathways to promote contrasting biological end points such as apoptosis, proliferation or differentiation, depending on the context[33]. Six driver kinases, which include the tumour suppressor serine/threonine kinase 11 (STK11) and the oncogene AKT1, can be grouped together based on their role in PI3K–mTOR signalling to cell proliferation, survival and protein synthesis (FIG. 2d). However, it should be noted that two additional mechanisms for dysregulation of this pathway in cancer are mutational activation of PIK3CA, the catalytic subunit of the lipid kinase PI3K, and inactivation or loss of PTEN. The cell cycle control and DNA damage signalling pathways are also well represented (FIG. 2g,h), with the repertoire of oncogenes and tumour suppressor kinases extended beyond well-characterized examples, such as cyclin-dependent kinase 4 (CDK4), ataxia telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3-related protein (ATR), to include never in mitosis A-related kinase 9 (NEK9), the functions of which include regulation of mitotic progression and the replication stress response pathway[34]. Two small subgroups of driver kinases remain. Kinases in the first subgroup act as key regulators of the actin cytoskeleton (FIG. 2f), and include TRIO, which positively regulates invasion and metastasis[35], and doublecortin-like kinase 1 (DCLK1), a marker of tumour stem cells in the intestine[36]. The second subgroup functions in transcriptional control (FIG. 2i), and includes CDK12, which positively regulates the expression of genes involved in DNA repair[37,38], and TATA box binding protein-associated factor 1 (TAF1), the largest subunit of the TFIID transcription factor complex[39]. Consistent with its oncogenic role, known roles for TAF1 include negative regulation of p53-dependent transcription via phosphorylation of p53 (REF. 40), promotion of cyclin D1 (*CCND1*) and *CCNA2* transcription[41], and co-activation of the androgen receptor[42].

Importantly, although two of the studies used to source these data assigned probable oncogenic or tumour suppressor roles for particular cancer drivers[27,28], assignment of these roles for many other kinases required additional literature surveys, which sometimes revealed contrasting results, depending on cancer type. In addition, our survey identified a substantial number of poorly characterized protein kinases with under-appreciated roles in cancer (Supplementary information S1 (table)). Twelve kinase drivers cannot be assigned readily to specific pathways or processes, including alpha-protein kinase 2 (ALPK2), casein kinase 1 epsilon (CSNK1E), CSNK1G3, CSNK2A1, leucine-rich repeat kinase 2 (LRRK2), SIK family kinase 3 (SIK3) and the lysine-deficient kinase WNK1. Others, such as MAP3K11 and MAP3K4, can be assigned to particular pathways, but the exact functional role of cancer-associated genetic alterations requires further clarification and is likely to be context dependent. Consequently, for many driver kinases, additional functional validation is required to confirm their oncogenic role and the effect of specific mutations on kinase activity, as well as other biochemical and signalling properties.

Therapeutic targeting of driver kinases. We have also evaluated the development status of therapeutic targeting for specific protein kinases in our driver list (FIG. 3; Supplementary information S3 (table), Synapse platform, Accession No. syn4961550). Of these kinases, 20 of 91 (22%) represent US Food and Drug Administration (FDA)-approved therapeutic targets. This subset comprises almost exclusively tyrosine kinases. A similar number of driver kinases (25%, exclusive of the first subset) are being targeted by agents in clinical trials. This subset

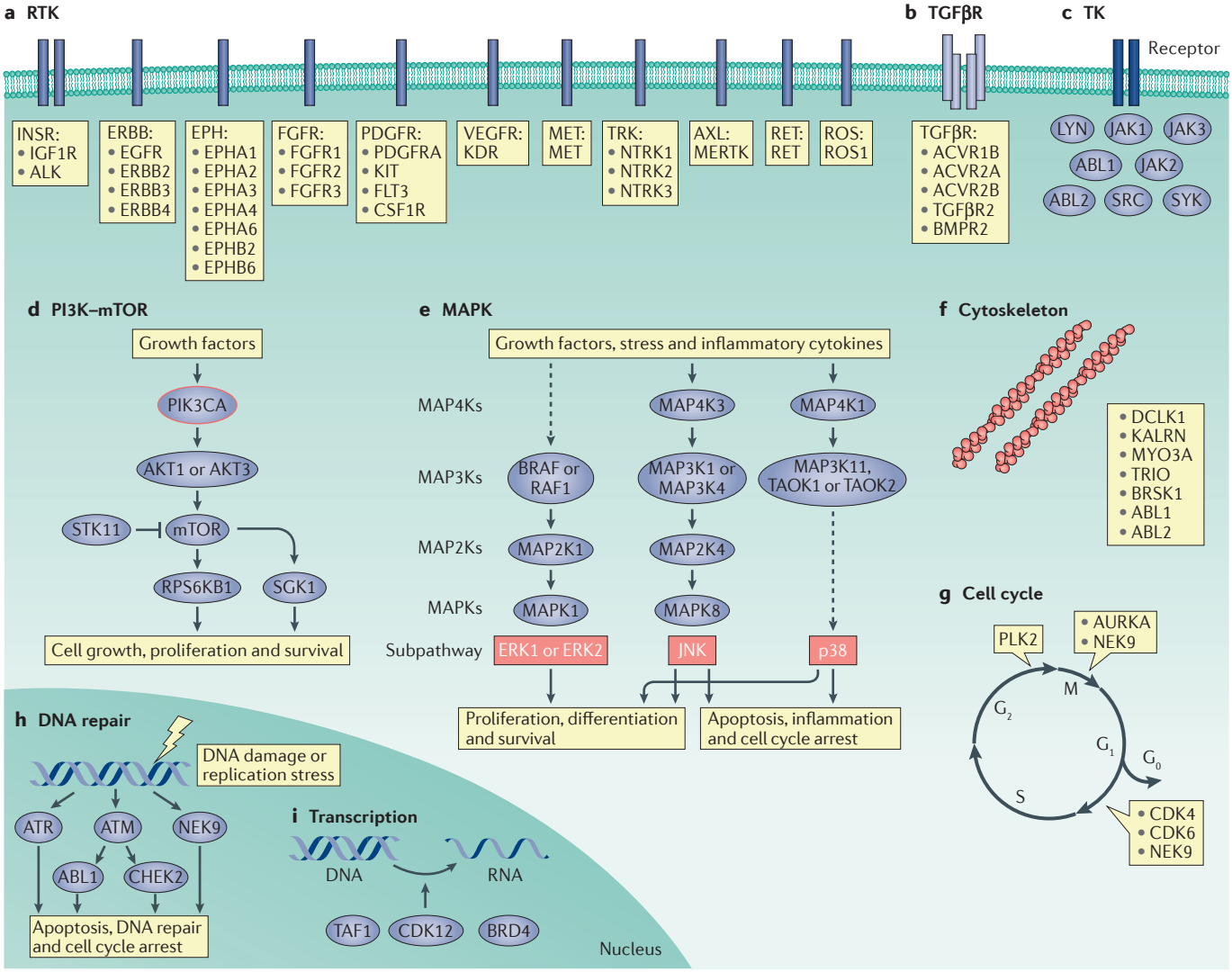
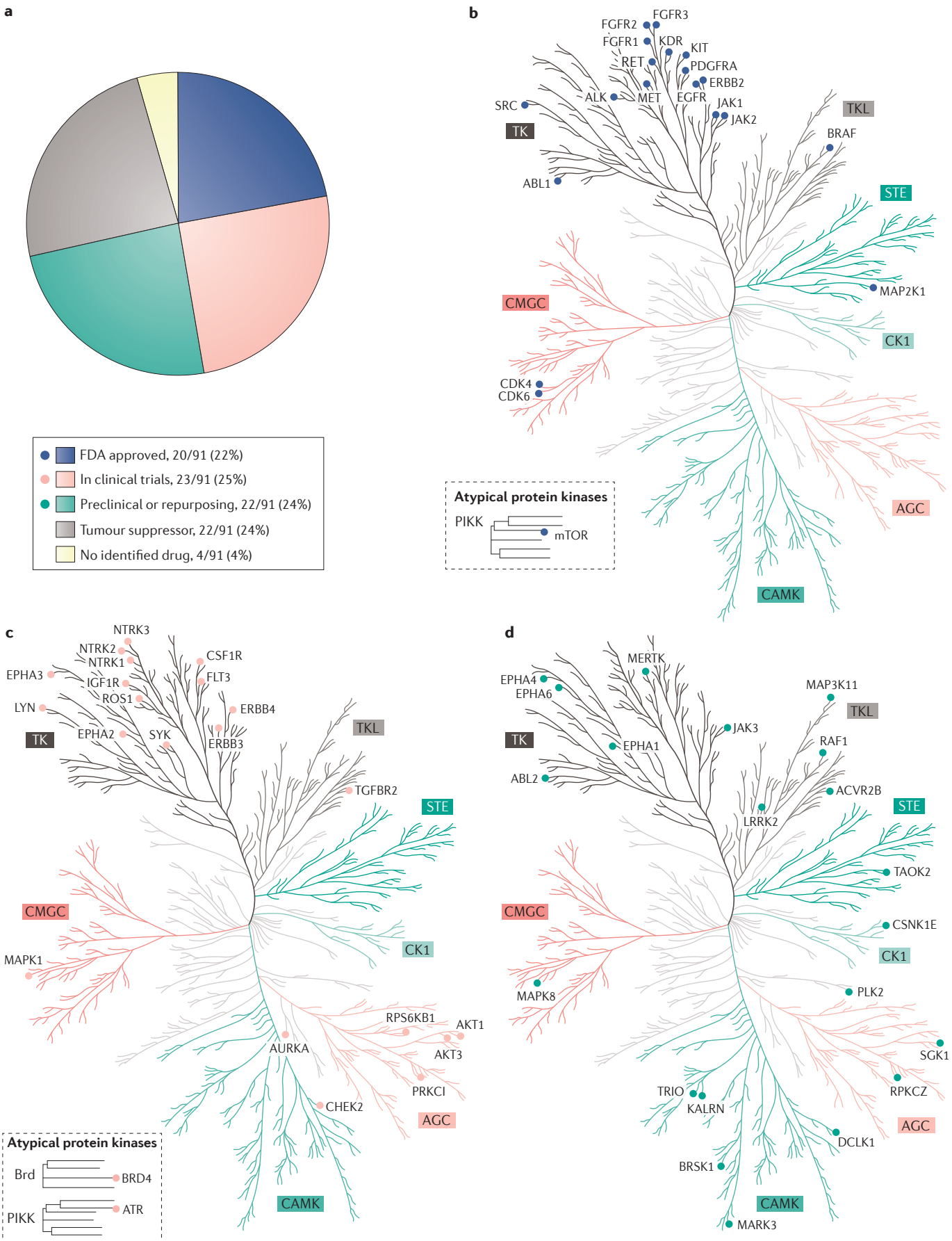


Figure 2 | **Assignment of driver kinases to core cellular pathways and processes.** This figure was compiled using information provided in Genecards and the literature. Names identified in blue ovals indicate particular driver kinases. Dashed lines indicate that the specific kinases involved at these steps in the pathway have not been identified as drivers. Assignment of driver receptor tyrosine kinases (RTKs) to particular RTK families (part **a**). Driver kinases that act as receptors for specific transforming growth factor-β (TGFβ) superfamily members (TGFβRs) (part **b**). Non-receptor tyrosine kinases (TKs) that act as drivers, highlighting their role downstream of particular types of cell surface receptor (part **c**). Particular driver kinases are also associated with the PI3K–mTOR (part **d**) and MAPK (part **e**) pathways, regulate cytoskeletal organization (part **f**) or the cell cycle (part **g**), or function in DNA repair responses (part **h**) or gene transcription (part **i**). Fifteen driver kinases are not included in the figure, either because their functional role requires further clarification (alpha-protein kinase 2 (ALPK2), casein kinase 1 epsilon (CSNK1E), CSNK1G3, CSNK2A1, leucine-rich repeat kinase 2 (LRRK2), protein kinase C iota (PRKCI), PRKCZ, SIK3, WNK1, DYRK1A, MARK3 and STK19), or because their associated pathway or process is not represented on the figure. The latter kinases are EIF2AK3, STK4 and TNIK. For further details see Supplementary information S1 (table). PIK3CA is the catalytic subunit of PI3K, and represents a lipid kinase commonly mutated in several human cancers. This is indicated with a red outline to differentiate PI3K from the protein kinases elsewhere in the figure.

is also dominated by tyrosine kinases but has substantial representation from serine/threonine kinases (FIG. 3). For the third subset (which comprised 24% of the 91 kinases), we sought to identify driver kinases that present opportunities for repurposing of approved therapies or those that are in clinical trials, or that are targeted by drugs or biologics in preclinical development. For this subset, we excluded protein kinases with probable tumour suppressor roles, unless there is a clear rationale for direct targeting (for example, to increase DNA damage). Consequently, the majority of the driver kinases, and almost all of the oncogenic drivers, exhibit corresponding therapeutic strategies at different stages of clinical development. However, our surveys highlight a small number of 'orphan' oncogenic kinases (GSNK1G3, MAP4K1, MYO3A and WNK1) for which there are no current targeting approaches as candidates for future drug development. There is also a need to



develop therapeutic strategies that exploit the vulnerabilities conferred by loss-of-function mutations in various tumour suppressor kinases, which could be addressed by functional genomic approaches, as discussed below.

A twist in the tail? Our list of kinase drivers is derived largely from studies aimed at identification of genes with patterns of mutation in human cancers that highlight them as likely oncogenes or tumour suppressor genes. However, two recent studies[43,44] indicate that this approach may not detect all protein kinases affected by driver mutations. The first characterized the effect of cancer-associated mutations in different members of the protein kinase C (PKC) family, which exhibit mutation rates of >10% in various human malignancies, including melanoma and pancreatic, stomach, colorectal and lung squamous cell carcinomas[43]. Functional analysis of 46 cancer-associated mutations revealed that 61% conferred loss of function, whereas the remainder had no effect. The loss-of-function mutations occurred across all PKC subgroups, raising the question of why conventional (for example, PRKCA) and novel (for example, PRKCD) PKC isozymes are not present in our driver list. Possible explanations include a relatively low proportion of truncating mutations in PKC isozymes that would lead to their classification as tumour suppressor genes[28], coupled with the lack of mutational 'hot spots' within the coding region, so that the corresponding genes are not highlighted as strong drivers by the algorithms or scoring approaches used in the primary data analysis articles[27,28]. Indeed, both PRKCD and PRKCE were highlighted as candidate tumour suppressors by Davoli *et al.*[27] but did not rank in their most stringent list, which we used in our analysis.

The second study developed a computational platform to identify cancer-associated mutations predicted to perturb signalling networks through different mechanisms[44]. This approach identified rare cancer-associated mutations in diverse protein kinases that may result in downstream 'rewiring' of signalling by altering kinase substrate selectivity, or that alter kinase signal output through constitutive kinase activation or inactivation. In the case of PRKCG and protein kinase D1 (PRKD1), the effect of specific mutations on kinase substrate selectivity *in vitro* was validated experimentally[44]. This work raises the intriguing possibility that non-recurrent kinase mutations confer context-specific effects on signalling networks and are of functional significance to the cancer cell in which they arise. Consequently, although our census provides a relatively high-confidence list of kinase drivers, it is likely that a greater proportion of the kinome is subject to functionally important mutational events, occurring at low frequency in a manner dependent on network context.

Insights from proteomics

Although genomic and transcriptomic approaches have identified many driver kinases in human cancer, the picture they paint is incomplete. This is because protein kinases are regulated at multiple levels, including protein translation, stability and post-translational modification, presenting further mechanisms for dysregulation in cancer that are not revealed by interrogation of samples at the DNA or mRNA level. Consequently DNA- or mRNA-based studies can result in false negatives by not identifying protein kinases dysregulated at one or more post-transcriptional levels, and false positives — for example, mutated kinases with low protein stability or kinases that are not overexpressed upon cognate gene amplification. A further confounding issue is that protein kinases usually function as components of larger pathways and networks, in which signal output can be subject to control mechanisms that act on separate pathway or network components. For example, dual-specificity phosphatase 4 (DUSP4) can act as a negative feedback regulator of nuclear ERK activation in cells expressing oncogenic BRAF[45]. Consequently, pathway and network activity also needs to be taken into consideration when assessing the potential driver role of a given protein kinase. Importantly, these shortcomings can be addressed using proteomics. Below, we compare and contrast the insights these methodologies provide with those described above for genomic approaches.

Antibody-based technologies. Reverse phase protein arrays (RPPAs) enable quantification of the expression and modification of relatively large numbers of proteins, typically 150–200, across hundreds or even thousands of samples, and have been applied to particular cancer types, such as breast cancer[21], and in pan-cancer studies[46]. When applied to breast cancer, RPPAs revealed that activation of AKT, a key effector kinase in the PI3K pathway, was not elevated in PIK3CA-mutated luminal A cancers, as might have been expected[21]. Instead, it was increased in the HER2 and basal breast cancer mRNA subtypes, where it correlated with loss of *PTEN* and *INPP4B* (encodes inositol polyphosphate 4-phosphatase type II), and *PIK3CA* amplification. Furthermore, integration of RPPA and mRNA expression data enabled subclassification of HER2-positive breast cancers into two subtypes, one with a HER2 mRNA subtype exhibiting high expression of EGFR, phosphorylated EGFR (pEGFR), HER2, pHER2 and pSRC, and another with mRNA and protein signatures characteristic of luminal subtypes[21]. These findings highlight how interrogation of protein kinases at the level of protein expression

Reverse phase protein arrays
(RPPAs). Microarrays carrying large numbers of protein samples printed as individual spots, with detection of particular antigens achieved via incubation with a specific antibody.

Breast cancer mRNA subtypes
Breast cancer can be subclassified into four major subtypes through gene expression profiling: luminal A, luminal B, HER2 and basal.

Inositol polyphosphate 4-phosphatase type II
A phosphatidylinositol 3,4-bisphosphate 4-phosphatase that antagonizes the PI3K signalling pathway and represents a tumour suppressor.

◀ Figure 3 | **Status of therapeutic development for driver kinases. a** | The proportion of driver kinases in each category of therapeutic development. **b** | Driver kinases targeted by US Food and Drug Administration (FDA)-approved therapies, with their position on the kinome tree highlighted by a blue circle. **c** | Targets of kinase inhibitors or antibodies that are currently in clinical trials in cancer patients, with targeted kinases indicated by a pink circle. **d** | Kinases that could be targeted by repurposing of approved therapies or therapies in clinical trials, or that are targeted by therapies in preclinical development are indicated by a turquoise circle. The main kinase groups (including 'atypical') are highlighted: AGC (containing protein kinases A, G and C); CAMK (calcium/calmodulin-dependent protein kinase); CK1 (casein kinase 1); CMGC (containing cyclin-dependent kinase, MAPK, glycogen synthase kinase 3 and CDC2-like); STE (homologues of yeast sterile 7, sterile 11 and sterile 20); TK (tyrosine kinase); TKL (tyrosine kinase-like).Kinome tree modified courtesy of Cell Signalling Technology, Inc. (www.cellsignal.com) and annotated using Kinome Render[102]. Only atypical protein kinases relevant to the corresponding panel are presented, because of space constraints. For details, see Supplementary information S3 (table).

or activation can provide unexpected insights into regulation of pathway activation and, because the two subtypes of HER2-positive breast cancers may respond differently to the HER2 antibody trastuzumab, can identify potential biomarkers of response to specific targeted therapies.

Application of the RPPA approach across 3,467 patient samples from 11 different cancer types provided further important insights into protein kinase activation in cancer[46]. In particular, this revealed that HER2 protein was increased more frequently in serous endometrial cancers than in breast cancer, whereas in bladder carcinoma, colorectal cancer and lung adenocarcinoma the frequency of HER2 protein overexpression was higher than that expected based on *HER2* copy number and mRNA expression. This identifies additional cancers that might benefit from anti-HER2 therapies[46]. In addition, reduction of tissue-specific signals in the RPPA data followed by unsupervised clustering revealed novel cancer subgroups that exhibited increased activation of targetable kinases, such as pAKT, pMAPK1 and pMAPK3. Focused analyses of specific pathways and actionable targets identified increased activation of specific kinases in certain cancer types, such as SRC in head and neck squamous cell carcinoma[46].

As an alternative to RPPAs, antibody microarrays can also provide quantitative data on the expression and phosphorylation of hundreds of protein kinases and signalling proteins across tumour lysate panels. In breast cancer, antibody microarrays enabled subclassification of triple-negative breast cancer (TNBC) specimens into four subgroups depending on protein expression and phosphorylation profiles, and identified MAPK7 (also known as ERK5) as a potential therapeutic target[47].

Mass spectrometry-based proteomics. The limitation of RPPAs and antibody microarrays is that they provide a limited proteome coverage restricted by the availability of highly selective antibodies. By contrast, mass spectrometry (MS)-based approaches enable global analysis of the proteome, or particular subproteomes defined by post-translational modification or binding properties, providing the capability to interrogate protein kinase expression or activation in cancer in an unbiased fashion. The power of this approach is highlighted by a recent study that used liquid chromatography–tandem mass spectrometry (LC–MS/MS) to characterize the proteomes of 95 colorectal cancers previously subjected to genomic and transcriptomic analyses by TCGA[48]. This revealed that mRNA abundance did not consistently predict protein abundance and enabled prioritization of candidate driver genes for particular amplicons based on corresponding increases in protein expression. In the context of protein kinases, this identified *SRC* as a candidate driver for the chromosome 20q amplicon.

However, the low abundance of many protein kinases limits the ability of MS-based proteomics to detect their expression, as well as their post-translational modification, unless different enrichment procedures are employed before LC–MS/MS. A widely used approach is purification of phosphorylated peptides using metal or metal oxide affinity chromatography[49]. As protein kinases themselves are often regulated by phosphorylation — for example, on the 'activation loop' of the catalytic domain — this approach can provide insights into the activation status of these enzymes. For example, when used to compare primary pancreatic ductal adenocarcinomas (PAADs) relative to normal tissue, this strategy detected increased phosphorylation of FYN on S21, a site that positively regulates the activity of the enzyme, in several cancer specimens, as well as significantly enhanced phosphorylation of CDC42-binding protein kinase-α (CDC42BPA; also known as MRCKα), AP2-associated kinase 1 (AAK1), MAP2K2 (also known as MEK2), homeodomain-interacting protein kinase 1 (HIPK1), p21-activated kinase 4 (PAK4) and MAP3K7 (also known as TAK1) in cancer tissue, albeit on uncharacterized sites[50]. In addition, phosphoproteomic profiling of BRAF-V600E$^+$ thyroid carcinoma cells following treatment with the BRAF inhibitor vemurafenib or the MEK inhibitor selumetinib detected increased abundance of phosphopeptides, representing substrates for acidophilic kinases, thereby identifying casein kinase 2 activation as a survival adaptation to blockade of BRAF–MEK signalling[51]. The latter example highlights how MS-based phosphoproteomics can provide crucial insights into signalling network remodelling following drug treatment and can identify novel therapeutic strategies.

Although many cancer drivers are protein tyrosine kinases, tyrosine phosphorylation accounts for <1% of cellular phosphorylation events, and this is reflected in the low relative yield of tyrosine-phosphorylated, versus serine/threonine-phosphorylated, peptides following metal or metal oxide-based affinity purification strategies. Consequently, detailed mapping of tyrosine kinase signalling networks in cancer requires enrichment of tyrosine-phosphorylated peptides before LC–MS/MS, and this is usually undertaken by immunoaffinity purification using monoclonal anti-phosphotyrosine antibodies. As many tyrosine kinases, particularly RTKs, are tyrosine phosphorylated on multiple sites, and these modifications have key roles in kinase regulation and signal transduction, this approach can provide important insights into the activation status of particular tyrosine kinases in human cancers. In a landmark study, Rikova *et al.*[52] undertook MS-based phosphotyrosine profiling across a large panel of non-small-cell lung cancer (NSCLC) cell lines and tumours, revealing many tyrosine kinases that exhibited high levels of tyrosine phosphorylation in particular subsets of patients and that represent candidate oncogenic drivers. In addition, this enabled subclassification of the tumour panel into five subgroups, with one exhibiting a broad kinase activation profile, and others exhibiting a more selective pattern[52]. Tumours or cell lines harbouring *EGFR* mutations or amplifications, and novel anaplastic lymphoma receptor tyrosine kinase (*ALK*) and *ROS1* fusions were identified by virtue of elevated tyrosine phosphorylation of the corresponding tyrosine kinase, but many additional protein kinases that might act as oncogenic drivers or modulators of response to targeted therapy were identified, including PDGFRα, discoidin domain receptor tyrosine

Liquid chromatography–tandem mass spectrometry (LC–MS/MS). When applied to proteomics, the liquid chromatography fractionates the peptides present in a sample and the tandem MS determines peptide mass and then additional characteristics through fragmentation.

Metal or metal oxide affinity chromatography
A technique used to purify phosphopeptides that exploits their binding to metal ions (such as iron) or metal oxides (such as titanium dioxide).

Pseudokinase
A protein with a protein kinase-related domain that does not exhibit kinase activity owing to the absence of one or more conserved amino acid sequence motifs.

kinase 1 (DDR1), AXL and several non-receptor tyrosine kinases, including specific members of the SRC family[52]. The overlap, and differences, between the suite of protein kinases implicated in NSCLC through genomic approaches (Supplementary information S1 (table)) and tyrosine phosphorylation profiling[52], respectively, are summarized in FIG. 4. Of note, the detection of NSCLC subsets exhibiting SRC family kinase (SFK) activation by tyrosine phosphorylation profiling is potentially significant, given the demonstrated role of these kinases in mediating resistance to anti-EGFR therapies[53].

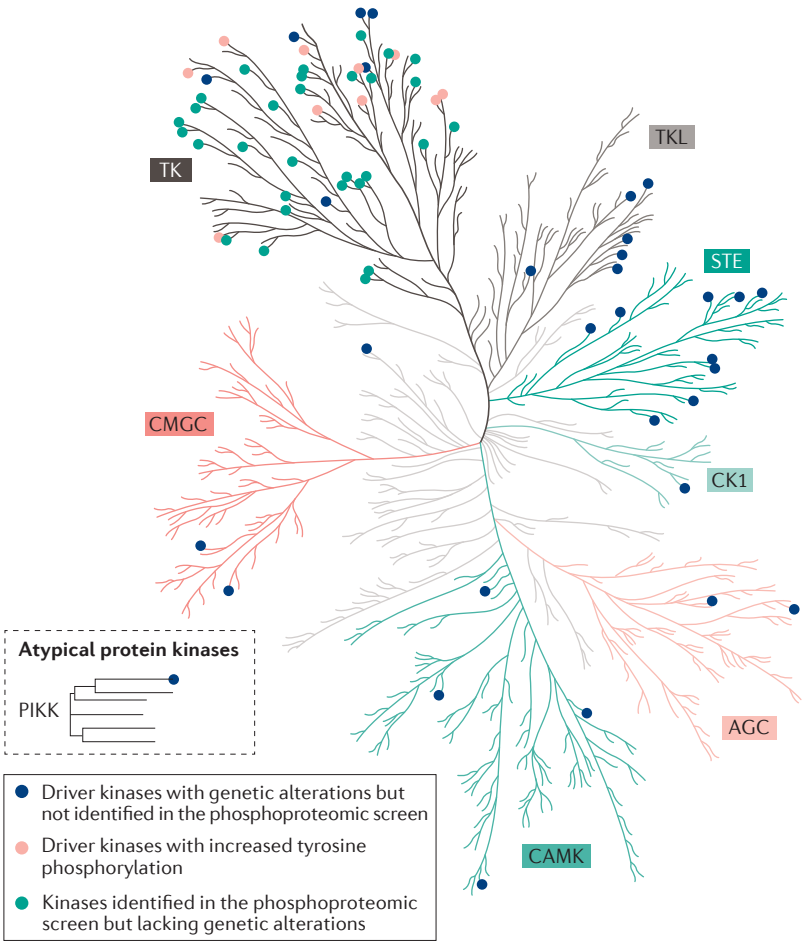


Figure 4 | **Comparison of deregulated protein kinases in non-small-cell lung cancer detected by genomic studies and phosphoproteomic profiling.** This analysis was undertaken by comparing driver kinases extracted from our cancer gene census (Supplementary information S1 (table)) with those exhibiting markedly enhanced tyrosine phosphorylation in this malignancy, as detected by mass spectrometry-based phosphoproteomic profiling[52]. Blue circles indicate driver kinases in non-small-cell lung cancer (NSCLC) identified through genetic alterations, but not highlighted by the phosphoproteomic screen; pink circles indicate driver kinases in NSCLC that also exhibit increased tyrosine phosphorylation; turquoise circles indicate protein kinases identified in the phosphoproteomic screen as exhibiting enhanced tyrosine phosphorylation, but lacking genetic alterations in NSCLC. The main kinase groups (including 'atypical') are highlighted: AGC (containing protein kinases A, G and C); CAMK (calcium/calmodulin-dependent protein kinase); CK1 (casein kinase 1); CMGC (containing cyclin-dependent kinase, MAPK, glycogen synthase kinase 3 and CDC2-like); STE (homologues of yeast sterile 7, sterile 11 and sterile 20); TK (tyrosine kinase); TKL (tyrosine kinase-like). Kinome tree modified courtesy of Cell Signalling Technology Inc. (www.cellsignal.com) and annotated using Kinome Render[102].

Phosphotyrosine profiling has also provided important insights into kinase deregulation in other cancers. For example, application of this approach identified PDGFRα as a potential therapeutic target in rhabdomyosarcoma, and insulin receptor (INSR) and IGF1R in subsets of Ewing sarcoma[54]. It also revealed that the basal breast cancer subtype is characterized by a signalling network governed by SFKs and exhibiting increased phosphorylation of multiple tyrosine kinases, including the RTK MET, LYN, EGFR, EPHA2 and focal adhesion kinase (FAK)[55]. The presence of this network had not been revealed by genomic or transcriptomic approaches (indeed, of the identified tyrosine kinases, only EGFR is listed as a breast cancer driver in Supplementary information S1 (table)), and as it was largely dependent on changes in tyrosine phosphorylation rather than protein expression, it would not have been resolved by analysis of the non-enriched proteome. Importantly, further dissection of this network via quantitative phosphoproteomics revealed an oncogenic signalling pathway involving the SFK LYN and the pseudokinase pseudopodium-enriched atypical kinase 1 (PEAK1)[56]. Tyrosine phosphorylation profiling can also reveal the tyrosine kinase signalling networks underpinning other types of phenotypic variation in cancer, such as sensitivity to specific chemotherapeutic or targeted drugs. For example, it led to the identification of FAK as a mediator of docetaxel resistance in castration-resistant prostate cancer[57], and of MET, AXL and SFKs as mediators of acquired resistance to EGFR tyrosine kinase inhibitors[53]. Comparable immunoaffinity purification strategies have now been developed for profiling other types of post-translational modification, such as ubiquitylation[58] and methylation[59], as well as sequence-selective phosphorylation events, such as those catalysed by ATM and ATR or AKT, RSK and S6 kinases[60,61]. Further application of these strategies is likely to reveal additional mechanisms whereby protein kinase function and downstream kinase signalling networks are perturbed in cancer.

A caveat to phosphorylation-based enrichment approaches is that the low cellular abundance of protein kinases can lead to an under-representation of this enzyme subclass upon MS analysis. This problem can be addressed by direct affinity purification of protein kinases using capture reagents such as combinations of broad-spectrum kinase inhibitors[62–64] or biotin-conjugated acyl nucleotide probes[65]. This strategy typically leads to subsequent detection of ~250 protein kinases by MS, representing the majority of the expressed kinome, and enables detailed interrogation of their phosphorylation status. An important application of this approach by the Johnson group has been characterization of the global impact of small-molecule targeted agents on the kinome over time, revealing extensive kinome reprogramming that limits therapeutic response[66,67]. In the first study, treatment of TNBCs with a small-molecule MEK inhibitor led to acute MAPK1 and MAPK3 inhibition that was followed by MEK2 and MAPK3 reactivation, reflecting enhanced expression of the RTKs PDGFRβ, DDR1 and vascular endothelial growth factor receptor 2 (VEGFR2)[66]. In the second, administration of the EGFR

NCI-60 cell line panel
A panel of 60 diverse human cancer cell lines used by the US National Cancer Institute to screen large numbers of chemical compounds, drugs and natural products for their biological activity.

and HER2 inhibitor lapatinib to HER2-positive breast cancer cells led to an adaptive kinome response involving increased expression and activation of diverse protein kinases[67]. In both cases, the drug-induced kinome reprogramming could be countered by a targeted strategy, involving administration of the multikinase inhibitor sorafenib in the first study[66] or small-molecule bromodomain and extraterminal motif (BET) inhibitors that block lapatinib-modulated gene expression in the second[67]. Of note, MS-based kinome profiling has been undertaken across the entire NCI-60 cell line panel, providing a valuable resource for the correlation of protein kinase expression and activation profiles with drug sensitivity[68].

Insights from functional genomics

Although characterizing the genetic alteration or dysregulation of protein kinases in a particular cancer may provide important insights into oncogenic mechanisms and can guide selection of candidates for further evaluation as therapeutic targets, these approaches do not, by themselves, reveal the dependency of the cancer on individual kinases. The importance of this issue is revealed by recent work on targeting of cancers harbouring the BRAF-V600E mutation with the small-molecule drug vemurafenib. Although this drug demonstrates marked efficacy against BRAF-V600E$^+$ melanoma cells in both experimental studies[69] and the clinical setting[3], it is less effective against colon cancers exhibiting this mutation[70,71], indicating that the presence of the BRAF-V600E mutation does not define sensitivity to the drug, and additional cell type-specific genetic or epigenetic factors also play a part. Importantly, although the influence of genetic background on 'kinase dependency' can present a clinical problem if this leads to drug resistance, it can also be exploited for development of novel therapeutic approaches if the genetic interactions are defined. This underscores the importance of identifying the functions of protein kinases not just in particular cell types but also in specific genetic backgrounds. As highlighted below, several different approaches have been used to address this issue, providing surprising insights into the dependency of cancer cells on specific protein kinases, and identifying that the landscape of such dependencies can be very different from that of kinase genetic alterations or expression and phosphorylation changes.

Cell-based RNA interference screens for 'essential' kinases. A powerful strategy for high-throughput interrogation of kinase function is to couple high-throughput small interfering RNA (siRNA) or short hairpin RNA (shRNA) screens encompassing either the whole genome or the kinome, with biological assays for relevant end points such as cell proliferation, viability or migration. An early study by the Harlow laboratory involving a shRNA screen across different human cell types determined that a given cell line was dependent on as many as 50–100 essential kinases (as assayed by continued cell proliferation and survival), with many of these representing poorly characterized enzymes[72]. In addition, aside from primary cell cultures from the same source or isogenic lines differing in the expression of a single gene, there were marked differences in kinase dependency between cell lines, even if they were from the same tissue type, with only 5% of the 278 essential kinases across four NSCLC cell lines being shared by all four. Consequently, although this study highlighted the untapped potential of the kinome for drug discovery[73], it also demonstrated the major impact of genetic background on kinase dependency. Importantly, subsequent studies exploited this relationship to reveal novel synthetic lethal interactions and thereby identify potential therapeutic approaches against cancer cells exhibiting oncogenic changes that are not directly druggable, such as loss of specific tumour suppressor genes, or challenging to current drug discovery pipelines, such as expression of activated RAS proteins (TABLE 1). This approach has also been used to identify kinases whose knockdown 'sensitizes' cells to specific therapies. Building on the identification of a synthetic lethal relationship between the tumour suppressors BRCA1 or BRCA2 and poly(ADP) ribose polymerase 1 (PARP1), which has led to the clinical testing of PARP1 and PARP2 inhibitors such as olaparib in BRCA1- or BRCA2-deficient cancers, a genome-wide shRNA screen in cells with wild-type BRCA1 and BRCA2 identified genes, including *CDK12*, that conferred sensitivity to PARP inhibition when silenced. As *CDK12* had been previously identified as a tumour suppressor in high-grade serous ovarian cancer (HGSOC) (REF. 74; Supplementary information S1 (table)) this work identified mutation or loss of CDK12 as a potential biomarker for PARP inhibitor sensitivity[75]. Furthermore, a shRNA-mediated kinome screen for kinases that sensitize colon cancer cells to BRAF inhibition identified EGFR as a mediator of vemurafenib resistance[71], leading to clinical trials involving combined therapeutic targeting of EGFR and BRAF in patients with colon cancer[76].

As an extension of this approach, several groups have undertaken functional genomic screening across large panels of cancer cell lines and integrated the resulting data sets with relevant genomic and transcriptomic information, enabling identification of the genetic vulnerabilities of particular cancer types and their molecular subclasses (TABLE 1). For example, shRNA screens across large panels of cell lines corresponding to different cancer types identified protein kinases that were classified as 'general essentials' — that is, required for proliferation of cell lines from different tissues — as well as 'tissue-specific' essential kinases[77,78]. In addition, a siRNA screen that focused on kinases and kinase-related genes across 34 breast cancer cell lines identified genetic dependencies for subsets of lines exhibiting distinct molecular characteristics[79]. In some cases, the protein kinases identified by these studies also exhibited corresponding gene copy number gains, and the combined functional and genomic data provide strong evidence for a driver role in that cancer. This applies to HER2 in subsets of breast and pancreatic cancer lines[78]. However, for others, such as TTK in PTEN-deficient cells, there is no corresponding mutation or CNA, and the cancer cell's vulnerability is hidden until unveiled

Table 1 | **Context-specific roles of protein kinases identified by functional genomics**

Background molecular characteristics or drug treatment	Kinase identified	Potential pathway or mechanism involved	Cancer type	Refs
Synthetic lethality or candidate genetic dependency				
Mutant KRAS	PLK1	Mitotic progression	Colon	111
Mutant KRAS	TBK1	NF-κB anti-apoptotic signalling	Lung	112
MYC or MYCN overexpression	BRD4	Regulation of *MYC* transcription	Ovary	83
MYC or MYCN overexpression	CSNK1E	Unspecified	Neuroblastoma	113
MYC amplification	AURKB	Unspecified	Lung (SCLC)	88
PIM1 overexpression	PLK1	Mitotic progression, MYC expression	Prostate	114
ER^+	ADCK2	Oestrogen signalling	Breast	79
ER^+ (hormone-independent)	PLK1	JUNB-dependent ER expression	Breast	115
$HER2^+$	TBK1	Regulation of p65–NF-κB signalling	Breast	116
$PTEN^{-/-}$	TTK	Mitotic checkpoint and aneuploidy	Breast	79
$PTEN^{-/-}$	NLK	Regulation of FOXO1	Several	117
$VHL^{-/-}$	CDK6, MET and MAP2K1	Unspecified	Kidney	118
p53 deficient	SGK2 and PAK3	Unspecified	Several	119
ERCC1 deficient	ATR	DNA damage signalling	Several	120
Drug sensitization				
Gemcitabine (nucleoside analogue)	CHK1	Unspecified	Pancreas	121
Cytarabine (nucleoside analogue)	WEE1	Unspecified	Myeloid and lymphoid leukaemias	122
Camptothecin (TOP1 inhibitor)	ATR	Inhibition of DNA elongation checkpoint	Breast and colon	123
Olaparib (PARP1 or PARP2)	CDK12	Suppression of DNA repair via homologous recombination, and reduced BRCA1 expression	Ovary	75
BRAF or ERK inhibition	ROCK1	Unspecified	Melanoma	104
Bevacizumab (VEGFA)	CHK1 and CHK2	Inhibition of HIF1α-induced DNA damage signalling pathway	Melanoma	124
Nutlin3 (p53 activation)	ATM and MET	Unspecified	Several	125
Selumetinib (MEK inhibition)	RAF1	Suppression of MEK inhibition-induced BRAF–RAF1 heterodimerization	KRAS-mutant colon and lung	126
Vemurafenib (BRAF-V600E inhibition)	EGFR	Suppression of adaptive EGFR signalling	Colon	71
MK1775 (WEE1 inhibition)	CHK1 and ATR	Cell cycle checkpoint interference	AML	127
Erlotinib (EGFR inhibition)	RPS6KA2	Regulation of RPS6	Pancreas	128

Protein kinases involved in synthetic lethal interactions, genetic dependencies or modulation of drug sensitivity identified through functional genomic or chemical screens. AML, acute myeloid leukaemia; ATM, ataxia telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related; AURKB, Aurora kinase B; BRD4, bromodomain-containing 4; CDK, cyclin-dependent kinase; CSNK1E, casein kinase 1 epsilon; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; ERCC1, excision repair cross-complementation group 1; FOXO1, forkhead box O1; HIF1α, hypoxia-inducible factor 1α; NF-κB, nuclear factor κB; PAK3, p21-activated kinase 3; PARP, poly(ADP-ribose) polymerase; PLK1, polo-like kinase 1; RPS6KA2, ribosomal protein S6 kinase polypeptide 2; SCLC, small-cell lung cancer; SGK, serum/glucocorticoid-regulated kinase; TBK1, TANK-binding kinase 1; TOP1, topoisomerase 1; TTK, phosphotyrosine picked threonine kinase; VEGFA, vascular endothelial growth factor A; VHL, Von Hippel–Lindau disease tumour suppressor.

by the functional genomic approach. In recognition of the power of this strategy to discover novel susceptibilities, Project Achilles, which aims to undertake genome-scale shRNA screens across hundreds of cancer cell lines with defined genomic and molecular characteristics, is under way[80], with the resulting data sets made available to the scientific community.

RNA interference screens for regulators of other cancer hallmarks. The majority of functional screens assay for effects on cell proliferation or viability. However, protein kinases regulate diverse cellular processes in cancer, and novel functional roles can be discovered by screening for modulation of alternative biological end points. For example, a siRNA screen identified ribosomal protein S6 kinase polypeptide 1 (RPS6KA1; also known as RSK1) as a novel suppressor of migration and invasion in lung cancer cells *in vitro*, and silencing of the RPS6KA1 homologue enhanced cancer cell metastasis in a zebrafish model. Subsequent analysis of patient specimens identified RPS6KA1 as a potential biomarker for metastasis in patients with lung cancer[81]. In addition, a functional screen for regulators of cell invasion identified PDGFRβ as a key driver of metastasis downstream

of mutant p53 in PAAD[82]. Expression of PDGFRβ positively correlated with poor disease-free survival of patients with PAAD, and importantly, treatment of mice harbouring PAAD tumours driven by activated KRAS and mutant p53 with imatinib, a small-molecule inhibitor of PDGFRβ activity, markedly attenuated metastasis, highlighting this drug as a potential therapeutic approach to blocking the spread of this devastating disease[82]. RNA interference (RNAi) screens can also be applied to *in vivo* models of human cancer. For example, screens in mouse models led to the identification of bromodomain-containing 4 (BRD4) as a potential therapeutic target in both HGSOC and AML[83,84]. Highlighting the novel insights provided by functional screens, the studies described in this section identified important dependencies on PDGFRβ or BRD4 that were not detected by genomic studies (Supplementary information S1 (table)), and whereas RPS6KA1 was identified as a fusion driver in lung cancer (Supplementary information S1 (table)), its role in cancer metastasis was only revealed by functional interrogation.

Importantly, CRISPR–Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR-associated 9) gene-editing technology provides a powerful alternative strategy for high-throughput interrogation of gene function that may be less susceptible to the off-target effects that often confound screens based on RNAi. A genome-wide screen for effects on tumour growth and metastasis using CRISPR–Cas9 has already been reported, indicating that this approach is likely to contribute substantially to future interrogation of the oncogenic roles of protein kinases[85].

Other types of functional screen. Although this section has focused so far on loss-of-function screens, gain-of-function screens can also provide important information regarding the role of specific kinases in mediating drug resistance. For example, expression of a library of open reading frame (ORF) clones representing ~75% of annotated kinases in a BRAF-V600E$^+$ melanoma cell line led to the identification of MAP3K8 (also known as COT) as a potential mediator of resistance to BRAF inhibition[86]. An alternative functional screening approach in mouse models uses insertional mutagenesis. For example, application of the Sleeping Beauty transposon system to a mutant KRAS-dependent mouse model of PAAD identified several protein kinases as candidate drivers for this malignancy, including RPS6KB1, CDK13, WNK2, MAP3K1, BMPR1A, MAPK1, FAK, ACVR2A and MAP2K4 (REF. 87). Of these, *ACVR2A* and *MAP2K4* were subsequently verified to be mutated in the human disease (Supplementary information S1 (table)), but it remains to be determined whether others are dysregulated at the transcriptional or post-transcriptional level.

Given the enzymatic nature of protein kinases, chemical library screens provide a powerful strategy for interrogating the role of particular kinases or subsets of the kinome in the mediation of particular cellular responses. A key advantage of such screens is that, as they can lead directly to identification of small-molecule compounds with biological activity against end points related to cancer development and progression, they are well positioned for rapid research translation. However, identification of the kinase targets of lead molecules usually necessitates integration with one or more omics-based screens. For example, combining data from genomic characterization of a large panel of small-cell lung cancer cell lines with results from a cell-based screen of 267 compounds identified a dependency on Aurora kinase B (AURKB) in cells with *MYC* amplification[88]. In addition, integration of chemical and RNAi screens highlighted glycogen synthase kinase 3α (GSK3α) and PI3K–mTOR as potential therapeutic targets in AML and osteosarcoma, respectively[89,90].

Summary and perspectives

Global interrogation at the genomic, proteomic and functional levels has provided major insights into dysregulation of the human kinome in cancer, as well as the role of specific kinases and the context-specific dependence of cancer cells on members of this enzyme superfamily. That said, the functional roles of many protein kinases that are mutated in cancer remain poorly characterized, and are often context dependent, varying according to cancer tissue type and genetic background within a single cancer. This problem is illustrated by the contrasting effects of loss-of-function mutations in particular protein kinases in human cancers. In the case of specific PKC family members, for example, these reflect an apparent tumour suppressor role[43]. However, kinase-impaired BRAF proteins can function as oncogenic drivers in the presence of active RAS by dimerizing with kinase-competent RAF1 (REF. 91). Consequently, despite the advent of high-throughput omics technologies, there is still the need for detailed biochemical and structural studies that probe the mechanism and regulation of identified kinase drivers. In addition, it is essential that functional studies are undertaken in appropriate cancer model systems that address the issue of genetic context. The tendency of the field to ignore this issue is highlighted by the frequent use of the MDA-MB-231 breast cancer cell line to model TNBC, despite the presence of activating KRAS and BRAF mutations that rarely occur in any type of breast cancer[92].

The identification of a census of high-confidence kinase drivers, and novel kinase dependencies defined by genetic background of cancer cells, provides novel kinase targets for drug development and repurposing (Supplementary information S3 (table)), and companion biomarkers to direct the use of targeted therapies. With regard to the stratification of patients for personalized treatment strategies, current genomic technologies enable not only identification of driver kinases that represent druggable targets in primary specimens, but also tracking of kinase-mediated acquired resistance to specific therapies through sequencing of circulating cell-free DNA[93,94]. However, as highlighted in this Analysis article, proteomic and phosphoproteomic techniques provide important information regarding the expression and activation status of protein kinases that is also relevant to the selection of appropriate therapies.

Sleeping Beauty transposon system
A technique that introduces a DNA vector at random sites throughout the mouse genome and thereby alters the expression of genes close to the insertion site.

RPPAs currently provide one antibody-based approach for relatively high-throughput proteomic analysis of clinical specimens, and recent developments in nanofluidic immunoassays provide the ability to detect phosphoproteins from as little as 20 ng of total protein, amounts consistent with those obtained from, for example, fine-needle aspirate biopsies[95]. In addition, targeted MS approaches involving selected reaction monitoring show great promise in terms of delivering the necessary specificity, sensitivity, reproducibility and throughput for routine analysis of clinical samples. Recent studies have provided 'proof of principle', demonstrating quantification of large numbers of protein kinases by this technique, either with[65] or without[96] prior affinity enrichment.

As summarized in FIG. 5, it is clear that the different omics technologies provide complementary information regarding the roles of particular kinases in certain cancers or oncogenic phenotypes. Reflecting this, a multipronged approach that uses more than one of these strategies will provide a more complete picture of kinase dysregulation and function. However, additional insights are often provided by integrating orthogonal data sets. This strategy, coupled with computational analysis, enables the inference of network models that provide valuable insights into cancer phenotypes and patient prognosis, and can be used to predict responses to particular perturbations, such as kinase mutation or drug treatment[97]. For example, integrating exome sequencing and RNAi data from breast cancer cell lines into a human signalling network reflecting regulatory and physical interactions identified subtype-specific cell survival networks, with AKT1 and SRC representing key network hubs for the luminal and basal subtypes, respectively[98]. In addition, network-based stratification, which enables clustering of patients according to the distribution of mutations over gene networks, identified distinct subtypes in ovarian, uterine and lung cancer, including a poor prognosis subtype in HGSOC characterized by a marked enrichment for genes in the FGF signalling pathway[99].

Inference of network models is also leading to the identification of novel treatment strategies that target the signalling network, rather than an individual protein, often referred to as 'network medicine'. In a landmark study published in 2012, systems-level interrogation of the response of TNBC to combined treatment with an EGFR inhibitor and doxorubicin identified that pre-administration of the EGFR inhibitor unmasked a pro-apoptotic pathway and markedly enhanced the efficacy of co-treatment[100]. Furthermore, in a study on tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in cancer cells[101], integrated functional, proteomic and phosphoproteomic screens ultimately enabled network modelling of information flow from apoptosis-modifying kinases and hence the identification of potential combination therapies to overcome TRAIL resistance.

Cancer genomes exhibit many genetic alterations that potentially have an impact on protein kinase signalling networks. Some of these directly affect protein kinase structure and/or expression, whereas others are

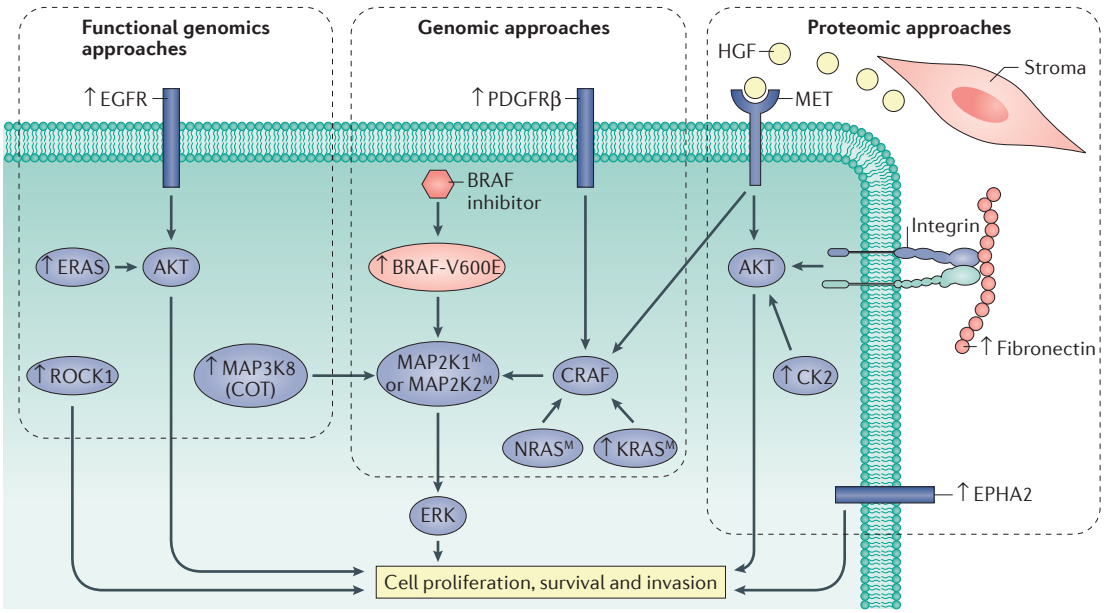


Figure 5 | **Different omics approaches provide complementary information regarding kinase function in cancer.** The figure summarizes our understanding of pathways regulating the behaviour of cancer cells expressing BRAF- V600E and exhibiting resistance to BRAF inhibition. It depicts pathways identified through functional genomics approaches[71,86,103,104] (left panel); genomic approaches[105–107] (middle panel) and proteomics approaches[51,108–110] (right panel). Vertical arrows indicate gene amplification, protein overexpression and/or activation. Superscript M indicates mutation. CK2, casein kinase 2; EGFR, epidermal growth factor receptor; EPHA2, EPH type A receptor 2; ERAS, embryonic stem cell-expressed RAS; HGF, hepatocyte growth factor; PDGFR, platelet-derived growth factor receptor; ROCK1, Rho-associated protein kinase 1.

likely to rewire networks by creating or ablating specific phosphorylation sites[44]. This apparent complexity presents a potential roadblock to gaining biological understanding and achieving effective clinical intervention. However, the insights already provided by different omics methodologies strongly suggest that their integrated use will overcome this challenge. For example, characterization of signalling networks associated with particular genetic backgrounds through MS-based analysis of phosphoprotein- or kinome-enriched fractions, followed by application of the same approaches to cells in which particular mutation patterns have been introduced or corrected by CRISPR–Cas9 technology, provides a potential strategy for understanding how such genetic alterations affect network behaviour. In parallel, functional screens can determine corresponding kinase dependencies. Ultimately, this will lead to computational models that predict the impact of specific mutational events as well as particular therapeutic interventions. As alterations in many different kinases may ultimately affect the same signalling network, this approach provides a pathway towards rational development of therapeutic strategies that target a limited number of crucial networks, in which the mutational or activation status of network components can be used to stratify patients for effective therapy.

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Competing interests statement
The authors declare no competing interests.

DATABASES
Catalogue of Somatic Mutations in Cancer database: http://cancer.sanger.ac.uk/cosmic

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